CENTER FOR DRUG EVALUATION AND RESEARCH

APPLICATION NUMBER: 021073

PHARMACOLOGY REVIEW(S)

JUN 30 1999

NDA 20-073

June 30, 1999

DRUG: Pioglitazone (ACTOS™)

INDICATION: NIDDM

TEAM LEADER MEMO TO FILE REGARDING PRECLINICAL PHARMACOLOGY/TOXICOLOGY ISSUES FOR NDA 20-073 (PIOGLITAZONE, ACTOS™)

The primary toxicity of concern with Pioglitazone is the increase in cardiac weight observed in the animal toxicology studies. This was a consistent finding in all species tested and occurred at low multiples of human exposure. The cardiac/hematological changes have been attributed to adaptive responses to plasma volume expansion and appear to be a class effect for thiazolidinediones. In long term rat and mouse studies (e.g., the 2-year carcinogenicity bioassays) the cause of death at high doses were attributed to cardiac effects.

A finding that is common for thiazolidinediones, increases in liver weights without histological correlates, was also observed in some animal studies (rats, mice, dogs and monkeys), but was not a consistent finding. There were no histological or clinical chemistry findings associated with liver weight increases in rats or mice. There were occasional instances of centrilobular hypertrophy noted in dogs and monkeys. Necrosis and subacute hepatitis was observed in one dog at approximately 50 times the human dose. Thus, although there is an increase in liver weight noted at fairly low multiples of human exposure, changes in histopathology or clinical chemistry are not noted until very high levels of exposure are reached. It is worth noting at this point that dogs appear to be the most sensitive species to the liver effects of thiazolidinediones.

Although this finding in the liver is not consistently found with pioglitazone, it is not possible based on the results of animal studies to say that pioglitazone is "safer" in this respect compared to other thiazolidinediones unless a head-to-head comparison is performed.

There are some points I would like to summarize to support the current labeling recommendations:

1. The mouse lymphoma reference originally included by the sponsor in the label as a negative finding was removed based upon the review of these data by the genetic toxicology committee. It was concluded that the standard assay with parent compound was inconclusive. It was not considered that the assay was adequately performed, therefore, the results should not be included in the label. In additional studies with the mouse lymphoma assay, there was a positive finding in the presence of metabolic activation with the M1 metabolite. This was reproducible and the assay was performed adequately. However, since this metabolite is not found in humans to a significant extent, it was thought that this was irrelevant and should not be included in the label. Findings in other permutations of the MLA were also deemed inconclusive by the committee. Based on the weight of evidence in other genetic toxicology studies which indicate that pioglitazone is not genotoxic, it was considered that the overall findings of the MLA are inconclusive and the MLA

findings should not be included in the label. Other studies indicating no genetic toxicity include the Ames bacterial mutagenesis assay, cytogenetics assays in the CHL, CHO/HPRT and AS52/XPRT models, an unscheduled DNA synthesis assay and an in vivo mouse micronucleus assay.

[redacted] This metabolite is found in humans and thus could be a possible concern. However, this information was based on an incomplete review. The genetic toxicology committee report indicates that the positive finding was with metabolite M1, which is not present in humans, not MIV.

3. In reference to the bladder tumors, although the proposed mechanism of mechanical irritation by calculi is plausible, there are not sufficient data to conclusively determine that this mechanism is wholly responsible for the bladder tumors observed in male rats [redacted]. However, given that the drug appears to be non-genotoxic and the drug itself did not directly induce a proliferative effect in the bladder in a BrdU incorporation experiment, further data may help to support this mechanism. It is of note that statistically significant tumors occurred only in male rats, although there were sporadic findings of benign tumors in female rats and a single occurrence of a bladder tumor in a high dose mouse. These were initially referred to in the label. [redacted] Currently, the relevance of these tumors in male rats to humans is unclear. The label is modified to reflect this. Note: the committee's summary of tumor findings was as follows:

   Mice: no significant tumor findings

   Rats: benign/and/or malignant transitional tumors in the urinary bladder in all dose-groups of the males except the lower dose. These were statistically significant by the trend tests.

4. The Pregnancy category was changed from the proposed "B" to "C" based upon the findings of embryotoxicity and delayed development. While there was maternal toxicity in some cases, this was considered to be relatively mild (change in maternal body weight compared to control <10%) and thus the fetal findings are significant. There was no teratogenic response to pioglitazone.

Based on the findings in the animal studies, it is difficult from a strictly Pharm/Tox standpoint to offer an unqualified approval to Pioglitazone. The toxicity of particular concern is the increase in heart weights, which is a consistent finding in all species tested. However, I believe that if clinical safety has been demonstrated in the data submitted to the NDA and such an assessment of safety is made by the reviewing medical officer, that this NDA should be approved. It is my understanding that the sponsor will be performing phase IV studies to examine this effect.

This reviewer recommends that this application is approvable (AE) from a pharm/tox standpoint pending a determination of clinical safety regarding the cardiac effects of pioglitazone.

/s/ [redacted] 6/30/99

Ronald W. Steigerwalt, Ph.D.
Pharmacology Team Leader

cc: NDA Arch
HFD510
HFD510/Steigerwalt/Misbin/HRhee/Weber
Review Code: AE (pending determination of clinical safety and labeling revisions)
Filename:TLmemo.doc

NDA 20-073 June 21, 1999

Takeda America Research and Development Center, Inc.
101 Carnegie Center, Suite 207
Princeton, New Jersey 08540

Submission: 2/23/1999 Received: February 26, 1999

REVIEW AND EVALUATION OF PHARMACOLOGY AND TOXICOLOGY DATA

Original Summary

Drug: Pioglitazone Hydrochloride(Actos™), AD-4833

Related: IND [redacted]
IND
IND

Pharmacological Class: Antidiabetic Agent

Indicated Use: Type II Diabetes

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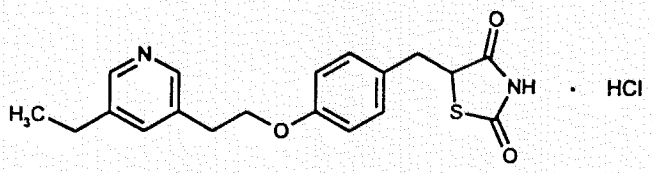
Pharmacological Class: Antidiabetic Agent

Indicated Use: Type II Diabetes

Clinical Dosage: 7.5 - 60 mg

Structure:



$C_{19}H_{20}N_2O_3S \cdot HCl$
MW = 356.43 + 36.57 = 392.90
(±)-5[[4-[2-(5-ethyl-2-pyridinyl)ethoxy]phenyl]methyl]-2,4-thiazolidinedione monohydrochloride

Foreign Studies: Yes – Please see individual studies.

1. INTRODUCTION

(The author would like to acknowledge the fact that this report contains materials directly reproduced from the sponsor's NDA reports. In cases where materials were directly reproduced, the reviewer has evaluated the data and agrees with the sponsor's presentation of the data. Points of disagreement are noted in this review).

One of the characteristic features of non-insulin-dependent (Type II) diabetes is insulin resistance where peripheral tissues have impaired glucose production and its utilization that leads to hyperglycemia. Pioglitazone hydrochloride [AD-4833(HCl) or [redacted] is an insulin sensitizer belonging to the class of thiazolidinediones. The exact mechanism of pioglitazone activity, as well as that of other thiazolidinediones, remains unclear. Pioglitazone decreases hepatic glucose output and increases insulin-dependent glucose disposal in skeletal muscle. Mechanistic studies indicate that thiazolidinediones act at many intracellular sites and can influence several processes to increase cell sensitivity to insulin. These include influence on insulin receptor kinase activity, change in number of insulin receptors, quantity and activity of GLUT-4, modulation of tumor necrosis factor (TNF-α) activity, activation of peroxisome proliferator-activated receptor-γ (PPARγ), and alteration of hepatic glucose metabolism.

2. PHARMACOLOGY AND PHARMACODYNAMIC STUDIES [redacted]

A. Antihyperglycemic Activities

Pioglitazone has been evaluated in a series of studies using normal, genetically obese, and diabetic (KKA^{y}) mice. Pioglitazone was incorporated into the diet such that the delivered doses were 2.4-24.5 mg/kg/day for four days. Blood glucose was decreased dose-dependently by 25% at a dose of 6 mg/kg/day and by 13% at 2.4 mg/kg/day. The drug also produced a dose-related decrease in plasma triglyceride and non-esterified fatty acids. In addition, the four-day treatment enhanced basal and insulin stimulated glucose oxidation and total lipid synthesis in adipocytes isolated from these mice.

When administered orally by gavage to genetically diabetic mice at doses from 6.25 to 100 mg/kg as a single dose, pioglitazone caused a significant reduction in blood glucose, and was sustained for two days. When administered in the diet in the same study, a dose of 25 mg/kg/day for four days was required to show hypoglycemic activity. Studies have also been conducted in the Wistar fatty rats because they can develop severe insulin resistance in peripheral tissues, such as muscle and adipose tissue, and liver. Pioglitazone administered orally to the rats at doses ranging from 0.3 to 3.0 mg/kg/day for up to one month produced a dose-dependent reduction in plasma glucose, plasma insulin, triglyceride and non-esterified fatty acids, with a dose of 0.5 mg/kg/day producing a 25% reduction in plasma glucose.

The calculated ED_{25} for pioglitazone base-induced plasma triglyceride lowering was 0.39

mg/kg/day and the ED_{25} for the HCl salt was 0.47 mg/kg/day. When adjusted for molecular weight, the HCl salt ED_{25} was 0.43 mg/kg/day. Thus, the pharmacological activities of both formulations were similar.

B. Pioglitazone Effect on Hepatic Tissues

In diabetic KKA^y mouse, treatment with pioglitazone for four days at 20 mg/kg/day decreased blood glucose, but also improved indices of hepatic insulin resistance. However, use of isolated liver cells by clamp techniques indicated that no alterations in phospholipase C activity were seen. In another study, pioglitazone significantly increased liver and brown fat catalase activity at doses ranging from 9 to 70 mg/kg in the KKA^y mouse model. It also increased liver peroxisomal β-oxidation in these mice at doses of 5.6 mg/kg/day and above.

In either genetically obese/diabetic Wistar fatty rats or in streptozotocin-induced diabetic rats, pioglitazone (3 and 10 mg/kg/day for seven days) decreased hepatic glucose production and increased peripheral glucose utilization in response to insulin treatment in the Wistar fatty rat . In streptozotocin-induced diabetic rats, pioglitazone (10 mg/kg for seven days) was also shown to improve hepatic insulin resistance in the presence of a minimal dose of insulin. In this same model, it was shown that the augmentation of insulin was accompanied by increased hepatic glucose-6-phosphate dehydrogenase activity and decreased glucose-6-phosphatase activity.

Using clamp techniques, pioglitazone increased the suppression of hepatic glucose production and peripheral glucose utilization to infused insulin. Pioglitazone also inhibited fatty acid metabolism and gluconeogenesis in isolated hepatocytes from starved Wistar rats. The subcellular mechanism of this effect is not yet clear, but presumably involves an alteration in the expression of those enzymes responsible for gluconeogenesis and possibly fatty acid metabolism.

C. Effects of Pioglitazone on Adipose Tissue

Although numerous studies were performed to identify the effect of the drug on adipose tissue, the outcome of these studies failed to identify a single mechanism of action. In *in vivo* studies, the administration of pioglitazone to diabetic KKA^y mice at doses of 0-71 mg/kg/day for six weeks resulted in a dose related increase in the adipocyte P2 gene (aP2) mRNA in epididymal fat. The aP2 gene is a specific gene implicated in adipocyte metabolism that codes for a protein involved in lipid transport. A similar effect was seen in perirenal fat, and correlated with a decrease in blood glucose. In the Zucker fatty rat, when treated for up to 28 days with pioglitazone at a dose of 10 mg/kg/day, weight gain was increased and glucose, insulin and triglyceride levels were decreased. Pioglitazone has been shown to upregulate the expression of the adipocyte fatty acid binding protein (aFABP) gene. This gene product is a substrate for insulin receptor tyrosine kinase and may be responsible for facilitating insulin signaling in adipocytes.

D. Peroxisome Proliferator-Activated Receptor Gamma

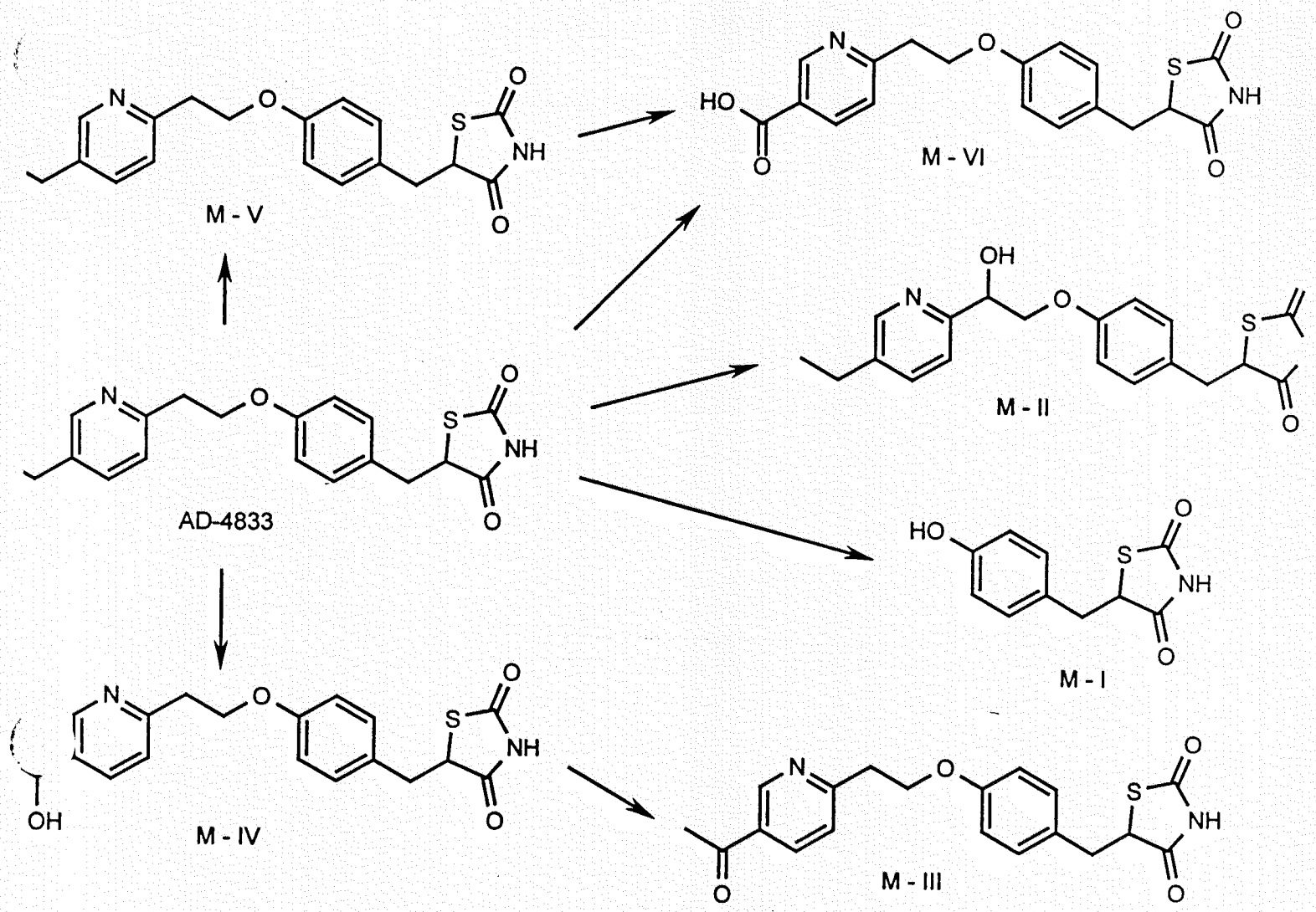
Peroxisome proliferator-activated receptor gamma (PPAR-γ) is part of the PPAR nuclear hormone receptor family. There are three identified subtypes of PPAR: α, δ, and γ, and possibly two subtypes of PPAR-γ. The tissue distribution of the PPARs varies, and PPAR-γ appears mainly in adipose tissue, but may be expressed in other tissues, including skeletal muscle. Initially, PPARs were found to be activated by compounds that caused proliferation of peroxisomes in the liver; subsequently, fatty acids, certain naturally occurring eicosanoids and thiazolidinediones were found to be activators of specific PPAR subtypes.

After binding and activation of PPAR-γ, the activated receptor complex may undergo a conformational change and bind to nuclear elements that regulate transcription of specific DNA sequences. One result of this interaction is differentiation of adipocytes from precursor cells. However, the mechanism of PPAR-induced adipocyte differentiation is not likely to be so simple, as other PPARs have been shown to be stimulated during adipocyte differentiation. Data indicate that specific, high-affinity binding of pioglitazone, to PPAR-γ causes its activation and results in adipocytes differentiation from fibroblasts. The differentiated adipocytes express specific genes coding for enzymes that are involved in lipid metabolism. The finding of increased food intake and increased adipose tissue weight in rats treated with thiazolidinediones provides some evidence of this mechanism *in vivo*.

It has been suggested that the drug induces adipocyte differentiation, and thereby promotes increased fat (triglyceride) uptake by the cells. The lipid lowering effect produced through increased fat uptake by adipose may result in improved glucose utilization by peripheral tissues, such as skeletal muscle. Through stimulation of triglyceride clearance by adipocytes, the skeletal muscle glycolytic pathway may be able to operate more effectively to reduce hyperglycemia. It has been also suggested that activation of PPAR-γ may alter cell production of paracrine or endocrine factor(s). Altered levels of these factors (and perhaps TNF-α) might reduce insulin resistance through yet unexplained mechanisms.

E. Pharmacological Activity of Metabolites of Pioglitazone

Pioglitazone is converted into six main metabolites (see the figure shown below). These metabolites have been evaluated for their hypoglycemic activity. Male Wistar fatty rats received an intraperitoneal injection of pioglitazone or one of its six metabolites (M-I through M-VI) for seven days. Pioglitazone, M-II, M-III and M-IV were injected at doses of 0.3, 1 and 3 mg/kg/day whereas M-I, M-V and M-VI were injected at a dose of 3 mg/kg/day. At the end of the treatment, plasma was collected for glucose and triglyceride determinations.



Pioglitazone(AD-4833) Metabolism and its Potential Metabolites

ED_{25} values were estimated for decreases in plasma glucose and triglyceride for each administered compound in fatty rats(See table below). Hypoglycemia and hypotriglyceridemia were only observed following dosing with pioglitazone, M-II, M-III and M-IV. Respective ED_{25} values for decreased glucose were 0.54, 0.99, 1.32 and 0.93 mg/kg and for decreased triglycerides were 0.43, 0.22, 0.47 and 0.53 mg/kg. These data confirm that most of the hypoglycemic and hypotriglyceridemic activity of pioglitazone is due to the parent compound .

Plasma Glucose

	AD-4833	M-I	M-II	M-III	M-IV	M-V
ED_{25} (mg/kg/day)	0.54	-*	0.99	1.32	0.93	>3.0
Relative Potency (AD-4833=1.0)	1.0	-	0.55	0.41	0.58	<0.18

Plasma triglyceride

	AD-4833	M-I	M-II	M-III	M-IV	M-V
ED_{25} (mg/kg/day)	0.43	-	0.22	0.47	0.53	>3.0
Relative Potency (AD-4833=1.0)	1.0	-	1.95	0.91	0.81	<0.18

*: Not determined

Since pioglitazone has an asymmetric carbon in its structure and two stereoisomers are known to be present, a study was conducted to evaluate the activity of the two stereoisomers. The potency of each stereoisomer was shown to be equal in potency to the racemate (pioglitazone). In an additonal study, the pharmacological activity of (±), (+), or (-) AD-4833 was studied in male Wistar fatty rats. Rats received oral doses of control material or 1 mg/kg/day of one isomer for seven days. All isomers decreased plasma glucose and triglyceride levels by 30 to 40% and 60 to 70%, respectively. These decreases were statistically significant and were not different between the isomeric formulations.

F. Cardiovascular Effects of Pioglitazone

Pioglitazone was evaluated for cardiovascular and hemodynamic effects in the anesthetized dog. The citric acid vehicle alone or the drug (1 and 3 mg/kg) was given intravenously, and evaluated for effects on mean arterial pressure, heart rate, cardiac output, left ventricular dP/dt/P, right atrial pressure, left ventricular end diastolic pressure, pulmonary artery pressure, and Lead II of the ECG. Cardiac index, stroke volume, and total peripheral resistance were calculated from the above parameters. Some effects were seen with the vehicle alone. The administration of pioglitazone did not produce any effects that were not seen with the vehicle. Therefore, while the data were influenced by the vehicle effects, there were no pronounced effects that could be attributed to the drug. Cardiac hypertrophy was also noted, but furosemide did not completely reverse it.

Cardiac hypertrophy was attributed to the increased plasma volume. In the same study, five day treatment with pioglitazone activated Na^+-K^+-ATPase in the proximal tubules and decreased Na^+ excretion in the urine, suggesting that the drug enhances the reabsorption of sodium and water. Cardiac enlargement was also produced by pioglitazone (10 and 100 mg/kg) in both hypophysectomized and intact rats, indicating that the effect is not dependent upon growth hormone. Thus, these studies indicate that the only major effect on the cardiovascular system is cardiac hypertrophy consequent to increased plasma volume.

3. ADME and PHARMACOKINETICS

AD-4833 was well absorbed from the gastrointestinal tract of mice, rats, dogs and monkeys with bioavailabilities ranging from 81% in mice and monkeys to 94% in dogs. Based on plasma AUC data for AD-4833 and its metabolites. Because AD-4833 is a racemate, possible enantiomer interconversion was studied and was found to occur both *in vitro* and *in vivo* with no changes in elimination. The addition of citric acid to the dose formulation produced a significant increase in bioavailability in both rats and dogs. In addition, slightly, but not statistically significantly higher absorption occurred with the hydrochloride salt of AD-4833 as compared to the acetic acid salt. The salt form did not affect half-life. Food appeared to slow absorption, but the effect was not significant.

A single oral dose resulted in slightly higher Cmax and AUC values in female rats than in male rats. Repeated dosing appeared to accentuate this response. Dose significantly affected the pharmacokinetics of AD-4833. Dose proportional increases in Cmax and AUC only occurred at low doses (*i.e.*, 4 mg/kg [repeated dose] or 7 mg/kg [single dose] and below in male rats and 16 mg/kg and below in female rats). Above these doses, increases in Cmax and AUC values were less than dose proportional. Treatment duration seemed to have no effect on AD-4833 pharmacokinetics in rats and dogs.

AD-4833 was highly bound to human serum albumin (*i.e.*, 97 to 98%). The drug was bound to a lesser extent to other serum proteins. Although AD-4833 could be displaced from its albumin binding site by several compounds, there was little or no displacement. Distribution studies demonstrated that AD-4833 is rapidly distributed to and eliminated from many tissues after single and repeated dosing. However, evidence of accumulation in and/or slow elimination from the fat (brown and epididymal) was observed. Administration to pregnant or lactating rats demonstrated that AD-4833 and its metabolites transfer to the fetus, milk and mammary glands.

Enzyme induction and inhibition studies were performed on AD-4833. AD-4833 was not found to inhibit or induce metabolic enzymes and did not change the microsomal or enzymatic protein content in hepatic microsomes. Although several cytochrome P450 isoforms are involved in the metabolism of AD-4833 to M-IV, CYP1A1 (an extrahepatic cytochrome), CYP2C8, and CYP3A4 are primarily involved. For M-II (a secondary metabolite), isoforms CYP2C9, CYP3A4, and CYP2C8 are involved. AD-4833 undergoes oxidative metabolism to form six metabolites, M-I through M-VI. M-IV is the

primary metabolite in human followed by M-III and M-II; these are pharmacologically active compounds. Although some differences exist between species in the relative amounts of each metabolite produced, metabolism occurs via the same routes.

The excretion of AD-4833 was investigated in mice, rats, dogs and monkeys. Fecal elimination predominated in all species, except monkeys where urinary elimination predominated. Repeated dosing in rats did not alter the elimination pattern. The following table summarizes the pharmacokinetic data. Unless otherwise specified, the route of administration was oral.

Summary of Pharmacokinetic Data

APK No.	Species	Dose	Tmax (hour)	Cmax (μg/mL)	AUC (0-48 hr) (μg*hr/mL)	Cumulative Excretion (%)	
						Urine	Feces
1	Mouse	0.5	1	0.51	3.00	23.9	74.9
2	Rat	0.5	4	0.71	7.11	35.5	61.7
	Dog	0.5	0.5	0.32	1.53	16.0	79.9
	Monkey	0.5	4.3	0.48	5.55	73.9	10.7
4*	Rat	5 (IV), 7, 107		--, 12.4, 106	80, 105, 1430	32.4, 41.4, 24.2	64.6, 48.8, 76.8
5**	Rat (pgmnt, albino)	1	4	1.5, 1.7	13.0, 13.6	NR	NR
9	Rat	10	1	13.62	107.47	NR	NR
13	Dog	10	1.4	8.73	26.99	NR	NR
14***	Dog	10	1-1.5	1.01-1.28	3.20-3.43	NR	NR
15	Dog	8	0.88	6.09	17.70 (0-7 hours)	NR	NR
16	Monkey	10, 20		1.23-4.29, 2.23-7.24		NR	NR
17	Dog (fasted)	25	1.12	15.44	53.46	NR	NR
18*	Rat	9.8	1.8	19.9	133	28.5 (f), 15.8 (m)	70.8 (f), 86.9 (m)
20*	Rat	10-300	1-4	3.17-12.68	39.12-181.47	NR	NR
21*	Rat	100-3000	4	12.53-13.59	171.78-230.20	NR	NR
22***	Dog	5-75	1-1.57	6.54-27.72	16.6-128.1	NR	NR
23*	Rat	0.5 (RD, day 7)	4.7	0.96	12.29	35.7	57.5

*AUC 0-24 hours; ** AUC 0-72 hours; *** AUC 0-∞ hours; IV = intravenous; f = female; m = male; pgmnt = pigmented.

In the mouse repeated dose study, less than dose proportional increases in plasma levels of AD-4833 and its three metabolites occurred. The overall trend in the repeated dose rat studies was for a less than dose proportional increase in Cmax and AUC values for AD-4833 and its three metabolites; some isolated greater than dose proportional findings were noted. In the 90-day and one year dog studies, increases in Cmax and AUC were less than dose proportional. However, in the 26-week dog study, mixed results were reported with greater than dose proportional responses in females and less than dose proportional increases in males. Monkeys exhibited a less than dose proportional increase in Cmax and AUC values following repeated dosing. In the mouse and rat carcinogenicity studies,

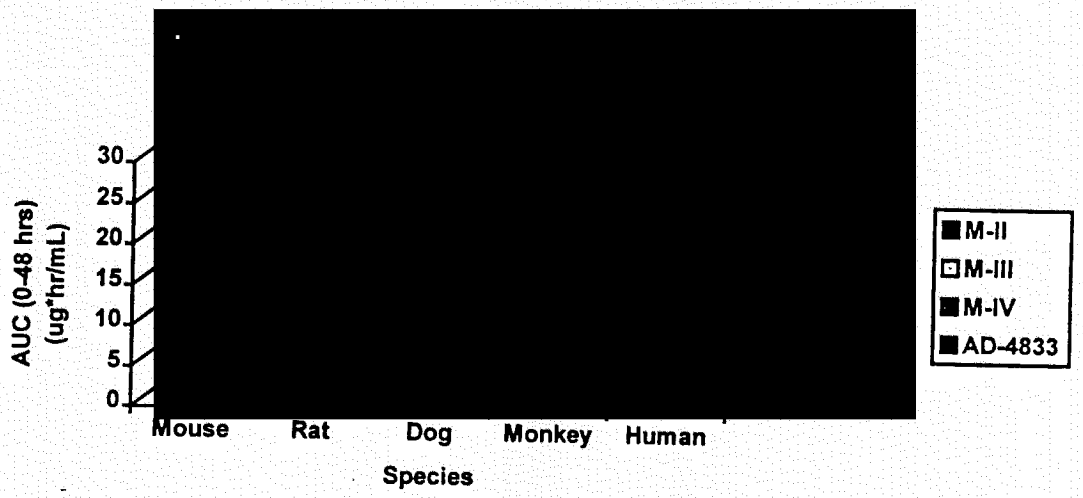
the trend was for a less than dose proportional increase in the Cmax and AUC values for AD-4833, M-II, M-III and M-IV. Isolated instances of greater than dose proportional increases were noted.

Although the effect of duration could not be assessed in mice, for the rats, increasing dose duration generally produced an increase in Cmax and AUC values for AD-4833 and/or the metabolites. In general, increasing duration produced increasing Cmax and AUC values in the dogs, but no consistent duration effect occurred in the monkeys. In all species, the duration effects were expressed at high doses; low doses did not induce this response. Isolated decreases in Cmax and/or AUC values with increasing dose duration were reported for the high-dose males in the mouse oncogenicity study, but no other duration effect was observed. In the rat oncogenicity study, increasing duration produced decreasing Cmax and/or AUC values at 63 mg/kg/day for AD-4833, M-II, M-III and M-IV.

No evidence of gender effects was observed in the mouse repeated dose study, but in the rat repeated dose studies females tended to have higher Cmax and AUC values than males. For dogs, no gender effects were evident overall, and no evidence of a gender effect was observed in monkeys for AD-4833. No gender effects were observed for AD-4833 or M-IV in the mouse oncogenicity study, but for M-II and M-III females had higher Cmax and AUC values than males. In comparison, in the rat oncogenicity study, females had consistently higher Cmax and AUC values.

To assess the relationship between animal and human exposures, a comparison was made between AUC values obtained at the NOEL dose in rats, dogs and monkeys with those obtained in a human clinical trial. Animal:human ratios of greater than 1 were obtained for rats and monkeys. A ratio of less than 1 was obtained for dogs as shown in fig. 2.

Figure 2. AUC Values of AD-4833 and Three Metabolites Following A Single Oral Dose



TOXICOLOGIC STUDIES(Most of all toxicological studies were performed by [redacted] In Japan under GLP standards, unless specificied otherwise)

A. Acute Oral Toxicity Study in Mice(T-2)

a. Methods: Crl:CF1[BR] mice (n = 3/sex/group) were given pioglitazone(AD-4833) orally at doses of 0, 337, 674 or 1738 mg/kg; free base equivalents of 0, 300, 600 and 1550 mg/kg, respectively. Clinical observations were performed for 11 to 12 days following the last dosing. A necropsy was performed on every animal. Histological examination was performed only on the organs which appeared abnormal during necropsy.

b. Results: The administration of the citrate-based granulated powder vehicle induced death due to gastric or gastrointestinal bloating. Clinical signs included wheezing, stomach distension and gasping, and occurred in all groups. Because the findings (clinical signs, mortality and pathology) in animals receiving AD-4833(HCl) were the same as those observed in animals receiving the vehicle alone, the acute toxicity of AD-4833(HCl) could not be defined in this study.

B. Acute Intraperitoneal Toxicity in Rat(T-1)

a. Methods: Five Jcl:Wistar rats/sex/group were injected pioglitazone by intraperitoneally at doses of 260, 360, 510, 710 and 1000 mg/kg.

b. Results: Decreased locomotor activity, respiratory depression and hyporeactivity were observed beginning 5 to 10 minutes after dosing in all groups. In addition, hypotonia of